Enzymes in Amniotic Fluid

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ABSTRACT

The determination of enzyme levels in cell-free amniotic fluid has proven useful in assessing fetal maturity and fetal well being, and is being utilized for the prenatal diagnosis of genetic disorders. The activities of amylase, α-galactosidase, phosphatidic acid phosphohydrolase, lysozyme and heat-stable alkaline phosphatase in amniotic fluid increase with gestational age and have an established relationship to fetal maturity. The ratio of amniotic fluid diamine oxidase activity to maternal serum activity (amniotic DAO/serum DAO) may be used as an indicator of the degree of rhesus isoimmunization after 28 weeks gestation. Creatine phosphokinase in amniotic fluid is elevated in cases of in utero fetal death and is of diagnostic significance. The prenatal diagnosis of Tay-Sachs disease, Sandhoff's disease, fucosidosis, GM1-gangliosidosis and I-cell disease have been made from the analysis of appropriate enzymes in cell-free amniotic fluid.

Introduction

Since the pioneering work of Bevis on the spectrophotometric analysis of amniotic fluid, many reports have appeared concerning the usefulness of various biochemical analyses of amniotic fluid in reducing perinatal mortality and morbidity. Some of these laboratory studies, aimed at ascertaining fetal status or the relationship of the fetus to its environment, have involved the determination of the activities of enzymes in the amniotic fluid.

The acceptance of transabdominal amniocentesis as a safe procedure and the development of more sensitive methods for enzyme analyses have made possible routine enzyme assays on amniotic fluid. Direct analysis of cell-free amniotic fluid has several advantages over methods which involve the growing of cells from the fluid in culture. In direct analysis, smaller volumes of amniotic fluid are usually required, the technical difficulties involved in cell culture are avoided and the results are available much sooner.

Some enzymes in amniotic fluid may result from the steady exchange of water and solute between intra-uterine compartments involving, among other less understood pathways, fetal swallowing and urinary excretion. Still others...
may be present in the fluid owing to the lysis of cells shed from fetal membranes. Even though the origin of most enzymes in amniotic fluid has not been thoroughly delineated, enzyme analysis of amniotic fluid has proven useful in assessing fetal maturity\textsuperscript{5,14,20} and fetal well being\textsuperscript{17,18,44} and is being utilized for the prenatal diagnosis of genetic disorders\textsuperscript{5,7,12,19,20,22,28}.

**Determination of Fetal Maturity**

During the latter half of pregnancy, the volume and composition of amniotic fluid change significantly. Some of the biochemical changes are sufficiently progressive and regular so that they may be utilized in the determination of the gestational age of the fetus and its well being. The osmolality of amniotic fluid and the concentration of creatinine, bilirubin, uric acid, and estriol have been related to the stage of gestation\textsuperscript{1,31,37} The concentrations of phospholipids have been shown to provide valuable information concerning fetal pulmonary maturity, thus making it possible to predict the susceptibility of a newborn to respiratory distress syndrome\textsuperscript{2,4}.

Many enzymes show a change in their activity at well defined times during gestation. The activities of amniotic fluid amylase\textsuperscript{5}, \(\alpha\)-galactosidase\textsuperscript{30} and phosphatidic acid phosphohydrolase\textsuperscript{14} as a function of fetal maturity have been studied. Their established relationship to fetal maturity is shown in figure 1.

**AMYLASE**

Amylase activities remain less than 200 I.U. in cell-free amniotic fluid until 36 weeks, at which time abrupt increases are observed\textsuperscript{5}. When a value of 250 I.U. was taken as indicative of fetal maturity, the incidence of false positives was 0.5 percent and that of false negatives was 38 percent. When the amylase activity was calculated per unit weight of total amniotic fluid protein and a value of 65 I.U. per g considered indicative of maturity, the incidence of false positives was 1.5 percent and the incidence of false negatives was 22.3 percent. This study included a large percentage of patients with toxemia, rhesus incompatibility and diabetes. Only one case is reported where these conditions affected the amniotic fluid amylase value. In this toxemic patient, an amylase activity of 834 I.U. was found at 31 weeks gestation. The lecithin/spingomyelin (L/S) ratio was 3.04, an indication of fetal maturity\textsuperscript{24}.

The source of amylase activity may be both the fetal pancreas and the salivary glands, and the route of transmission to the amniotic fluid may be the urine or saliva\textsuperscript{46}. This is supported by the finding that the isoenzyme pattern of amniotic fluid amylase changes with gestational age.
ENZYMES IN AMNIOTIC FLUID

fluid amylase resembles that of newborn urine. It has also been reported that the activity of amniotic fluid amylase was unaffected by maternal serum activities as high as 1600 I.U. in a patient with chronic pancreatitis and a pancreatic pseudocyst.

Amylase activity has been determined in the amniotic fluid, after separation of suspended cells by centrifugation, by either a classical procedure using starch as substrate or a test using a dye-labeled substrate.

α-GALACTOSIDASE

The activity of α-galactosidase in amniotic fluid has been shown to be a sensitive indicator of gestational age. The activity remains at less than 2 mU per g protein before 30 weeks after which it rises rapidly to a range of 8.2 ± 3.9 mU per g protein at term. Using an activity value of 2.7 mU per g protein as indicative of at least 33 weeks gestation, 89 percent of the patients at less than 33 weeks and 93 percent greater than 33 weeks gestation were correctly diagnosed. This criterion for gestational age was found to be correct in 60 of 66 cases studied. These results are consistent with those of a larger study which included women with rhesus isoimmunization.

The α-galactosidase activity can be determined fluorimetrically by measuring the liberation of 4-methylumbelliferone when using 4-methylumbelliferyl-α-D-galactoside as the substrate. The unit for α-galactosidase activity is defined as that amount of enzyme which hydrolyses 1 nmol of substrate per min at 37° (1 mU). When the ratio of enzymatic activity to the amniotic fluid total protein was used, the relative increase in activity at term was accentuated. After centrifugation of the fluid, the supernatant can be stored at 4° for two months or at −20° for two years with no alteration in activity.

PHOSPHATIDIC ACID

PHOSPHOHYDROLASE (PAPase)

PAPase is an enzyme which catalyzes the hydrolytic cleavage of phosphatidic acid to form 1, 2-diglycerides, which act as acceptors of phosphorylcholine in the formation of lecithin in the fetal lung. Since amniotic fluid is inhaled and exhaled by the fetus, this mechanism may be responsible for the transport of both PAPase and lecithin to the fluid surrounding the fetus. The specific activity of PAPase in amniotic fluid remains constant at less than 15 nmol of phosphate released per mg of protein per hour before 30 weeks gestation. The activity increases to its maximum, 100 nmol of phosphate released per mg per hour, at 37 weeks. This increase in activity has been shown to parallel the increase in L/S ratio but to begin earlier. When the value of the L/S ratio was found to be greater than 2, the level of PAPase activity was greater than 30 nmol phosphate released per mg protein per hour. The determination of this enzyme in the amniotic fluid may be of use in the evaluation of fetal lung maturation.

PAPase activity is assayed by incubation of phosphatidic acid with amniotic fluid at 37° for 40 min and by measuring the amount of inorganic phosphate liberated. The activity is defined as nmol of phosphohydroxylase from the phosphatidic acid per mg of total amniotic fluid protein per hour. The enzyme has maximal activity at pH 6.0, and its action is inhibited by Mg++, Be++, Tween 20 and KF. After collection, the samples were chilled in ice and centrifuged at 4°. The supernatant fraction has been stored up to three weeks at −20° with no loss in activity.

LYSOZYMES

The lysozyme activity in amniotic fluid obtained at the time of delivery of a normal full term infant was found to be in the
range of $9.3 \pm 3.0$ mg per 1 by diffusion on agarose-gel. The activity of lysozyme in amniotic fluid as a function of gestational age has been studied, and a progressive increase in absolute concentration up to 40 weeks gestation has been found. The mean value for lysozyme activity in patients with uncomplicated pregnancies between 14 and 19 weeks gestation was in the range of $5.1 \pm 1.7$ μg per ml and rose to $18.9 \pm 4.3$ μg per ml at 38 to 40 weeks gestation.

**Heat Stable Alkaline Phosphatase**

In normal pregnancy, there is an increase in the activity of heat stable alkaline phosphatase (HSAP) in amniotic fluid from a range of $1.71 \pm 0.185$ at 35 weeks gestation to a range of $2.60 \pm 0.329$ King-Armstrong units at 38 weeks. In pre-eclampsia, the activity of HSAP is significantly higher than normal in the amniotic fluid with a range of $2.71 \pm 0.491$ at 35 weeks and increasing to a range of $4.33 \pm 1.118$ King-Armstrong units at 38 weeks. A temperature of $56^\circ$ was used for the denaturation of “non-placental” alkaline phosphatase. Others report a better correlation if a temperature of $65^\circ$ is used for the denaturation.

**Rhesus Isoimmunization**

The activity of diamine oxidase (DAO) in amniotic fluid has been used in the assessment of fetal well being in pregnancy complicated by rhesus isoimmunization. In cell-free amniotic fluid from normal pregnancies, there is an increase in DAO activity from 40 mU per at 9 to 12 weeks gestation to a level of 6,000 mU per at 28 weeks. The activity remains at approximately this value after 28 weeks with only a slight decrease towards term. The activity of DAO in maternal serum also shows an increase from 200 mU per at 9 to 12 weeks gestation to 1,500 mU per at 25 weeks. The serum activity remains constant after 25 weeks and is less than the activity in the amniotic fluid.

The activities of DAO in both amniotic fluid and maternal serum from pregnancies complicated by rhesus isoimmunization are within normal ranges if the fetus is only mildly or moderately affected. In cases where the fetus is severely affected, the level of DAO activity in amniotic fluid shows a slight increase, but there is a large overlap with the normal range. The maternal serum level of DAO is usually within the normal range, as it is in most complications of pregnancy.

It was found, however, that the ratio of amniotic fluid DAO activity to maternal serum activity (amniotic DAO/serum DAO) after 28 weeks gestation was a significant indicator of the degree of rhesus isoimmunization. The value for this ratio in mild, moderate, and unaffected pregnancies was found to be in the range of $2.7 \pm 1.9$. The range found in severely affected pregnancies was $7.7 \pm 2.6$. The use of this ratio in conjunction with the Δ450 nm peak for bilirubin can provide additional information about fetal involvement in rhesus isoimmunized pregnancies after the 28th week of gestation.

A study of lactate dehydrogenase (LDH) activity and the ratio of the isoenzymes in amniotic fluid has been made in rhesus isoimmunized pregnancies. The results of this study indicate that there is no significant difference in either the total LDH activity or in the isoenzyme distribution between sensitized and non-sensitized pregnancies. These results are not consistent with an earlier report that the activity of LDH 5 was uniformly elevated in the amniotic fluid when the fetus was severely affected. A study of both acid phosphatase and alkaline phosphatase activities in sensitized pregnancies showed no significant changes of the activities in amniotic fluid from normal pregnancies. This is also true for all of the lysosomal enzymes thus far studied in amniotic fluid.
Fetal Death

The diagnosis of fetal death in utero has been made by the measurement of creatine kinase (CK) activity in amniotic fluid. Unlike the use of a sharp decrease in the estriol level in the maternal urine, this test is independent of gestational age and does not require serial 24-hour urine collections to confirm the diagnosis. The amniotic fluid CK activity is elevated above the normal value of 0 to 10 mU per ml (normal maternal serum activity: 15 to 145 mU per ml) within three days after fetal death. In a study of 247 normal pregnancies, there were no samples which exhibited an elevated CK level. The activity of CK in amniotic fluid in the case of fetal death showed a 10-fold to 180-fold increase; maternal serum activities were not elevated. This increase in amniotic fluid CK activity is apparently due to the release of the enzyme by decomposing fetal tissue.

In one case studied, it was found that an elevated level of LDH (2.5 times the upper limit of normal) in the presence of a normal CK activity foreshadowed fetal death. This was confirmed by repeat amniocentesis 12 days later, when a markedly elevated CK activity was found in the amniotic fluid. However, the use of LDH activities to diagnose fetal death is limited by the lack of specificity of this enzyme and the possibility of maternal erythrocyte contamination of the amniotic fluid sample.

Inborn Errors of Metabolism

The analysis of enzyme activities in cell-free amniotic fluid has been used in prenatal diagnosis of several metabolic diseases (table 1). The use of this technique has been criticized because the enzyme activity measured may not be specifically of fetal origin and, therefore, the activity may not reflect the true biochemical activity in the fetal tissues. Experience, however, has shown the usefulness of this procedure, and the following errors of metabolism have been successfully diagnosed in utero by enzyme analysis of cell-free amniotic fluid.

**TAY-SACHS DISEASE**

Tay-Sachs disease or \( \text{GM}_2 \)-gangliosidosis Type I is the most frequently oc-

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**TABLE I**

<table>
<thead>
<tr>
<th>Disease</th>
<th>Enzyme</th>
<th>Normal Activity</th>
<th>Affected Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tay-Sachs disease</strong></td>
<td>Hexosaminidase</td>
<td>Total: 658 nmol/hr/ml</td>
<td>Total: 529 nmol/hr/ml</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A: 4.2-35.6% total on acrylamide gel</td>
<td>A: none detected</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A: 5.7-32% total on heat fractionation</td>
<td>A: 8.1-10.2%</td>
</tr>
<tr>
<td><strong>Tay-Sachs disease</strong></td>
<td>Hexosaminidase</td>
<td>Total: 283-592 nmol/hr/ml</td>
<td>Total: 350-726 nmol/hr/ml</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A: 11-26% total</td>
<td>A: 3-8% total</td>
</tr>
<tr>
<td><strong>Sandhoff's disease</strong></td>
<td>Hexosaminidase</td>
<td>Total: 580-1020 nmol/hr/ml</td>
<td>Total: 12.5 nmol/hr/ml</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A: 11-30% total</td>
<td>A: 20%</td>
</tr>
<tr>
<td><strong>Fucosidosis</strong></td>
<td>( \alpha )-Fucosidase</td>
<td>41-48.4 nmol/hr/ml</td>
<td>3.0-5.5 nmol/hr/ml</td>
</tr>
<tr>
<td></td>
<td>( \beta )-Galactosidase</td>
<td>3.1-7.7 nmol/min/g</td>
<td>7.8-12.0 nmol/hr/ml</td>
</tr>
<tr>
<td><strong>Pompe's disease</strong></td>
<td>( \alpha )-1,4 Glucosidase</td>
<td>23.3-61.3 nmol/hr/ml</td>
<td>Unchanged</td>
</tr>
<tr>
<td><strong>GM_1-gangliosidosis</strong></td>
<td>( \beta )-Galactosidase</td>
<td>0.33 nmol/min/ml</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>( \beta )-Glucuronidase</td>
<td>0.2 nmol/min/ml</td>
<td>1.67 nmol/min/ml</td>
</tr>
<tr>
<td></td>
<td>Arylsulfatase A</td>
<td>0.06 nmol/min/ml</td>
<td>0.83 nmol/min/ml</td>
</tr>
<tr>
<td></td>
<td>( \alpha )-Galactosidase</td>
<td>0.003 nmol/min/ml</td>
<td>0.248 nmol/min/ml</td>
</tr>
<tr>
<td></td>
<td>Hexosaminidase</td>
<td>6.26 nmol/min/ml</td>
<td>0.016 nmol/min/ml</td>
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<tr>
<td></td>
<td>Acid phosphatase</td>
<td>0.92 nmol/min/ml</td>
<td>54.7 nmol/min/ml</td>
</tr>
<tr>
<td></td>
<td>( \alpha )-Glucosidase</td>
<td>1.13 nmol/min/ml</td>
<td>0.97 nmol/min/ml</td>
</tr>
</tbody>
</table>

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curring ganglioside storage disease known. The disease occurs predominantly among individuals of Ashkenazi-Jewish ancestry, and the number of known cases is in the thousands. The onset of symptoms occurs in the first six months of life and the disease runs a fatal course. The patient usually expires from bronchopneumonia by three years of age. The detection of heterozygous individuals by serum hexoaminidase assay is possible, and there is a need for a rapid method for in utero screening in high-risk pregnancies.

The enzymatic defect in Tay-Sachs disease is a deficiency in hexosaminidase A. This hexosaminidase component is separable from the B form by electrophoresis, by heat denaturation of hexosaminidase A, and by pH inactivation of hexosaminidase A. The prenatal diagnosis of Tay-Sachs disease by enzymatic assay of cell-free amniotic fluid has been made and compared with methods using both cultured and uncultured amniotic fluid cells. The reliability of diagnosis made using cell-free fluid has proved to be as good as those made from analysis of cultured cells. The difference in value between controls and affected fetuses is smaller for this method than that obtained with uncultured cells and is much less than that obtained with cultured cells. The use of acrylamide gel electrophoresis for fractionation of the hexosaminidase has been found to differentiate better between controls and affected fetuses.

In a study of 15 high-risk pregnancies, five diagnoses of Tay-Sachs disease were made in utero on the basis of deficient hexosaminidase A activity in the cell-free amniotic fluid. The total hexosaminidase activity was determined by measuring the amount of liberated 4-methylumbelliferone upon incubation of the amniotic fluid with 4-methylumbelliferyl-β-D-N-acetylglucosamine at 37°. The total activity of hexosaminidase in the affected pregnancies was normal or slightly elevated. The percentage of total activity owing to hexosaminidase A was determined by a heat denaturation method and found to be less than 8 percent (normal: 11 to 26 percent). If acrylamide gel electrophoresis is used to separate the hexosaminidase fractions, the gels can be quantitated by fluorimetric scanning after incubation with the 4-methylumbelliferyl-β-D-N-acetylglucosamine. By use of this method, no hexosaminidase A activity was detected in the cell-free amniotic fluid of affected fetuses.

The work that has been done on the prenatal diagnosis of Tay-Sachs disease seems to demonstrate that direct analysis of cell-free amniotic fluid is as reliable as other methods, and may be the method of choice as the diagnosis can be made on the same day as the amniocentesis is performed.

**Sandhoff's Disease**

Sandhoff's disease is a variant of Tay-Sachs disease which results from the lack of both β-N-acetyl-hexosaminidase A and B activities. The clinical course of the disease is similar to classical Tay-Sachs disease except that it has occurred only in families with no Jewish ancestry. The disease is diagnosed by finding a reduction of activity of both A and B isoenzymes to about 10 percent of normal in leukocytes or serum. The diagnosis of Sandhoff's disease has been made in utero by the demonstration of marked deficiencies of hexosaminidase A and B activities in cell-free amniotic fluid. In one case the total hexosaminidase activity in the affected pregnancy was only 12.5 nmol of 4-methylumbelliferyl-β-N-acetylglucosamine hydrolyzed per ml per hr compared to a control value mean of 842 nmol per
ml per hr. The diagnosis was confirmed after termination of the pregnancy by demonstration of deficient activity of both hexosaminidase A and B in the fetal tissues.

**G<sub>M1</sub>-Gangliosidosis**

G<sub>M1</sub>-gangliosidosis is an hereditary autosomal recessive mucopolysaccharidosis in which lysosomes are overloaded with compounds requiring β-galactosidase for their degradation. In tissue samples from patients with the disease, there is an overall decrease in β-galactosidase and α-L-arabinosidase activity. The activity of both of these enzymes in cell-free amniotic fluid from normal pregnancies has been reported.

The prenatal diagnosis of G<sub>M1</sub>-gangliosidosis has been made by showing the absence of β-galactosidase activity in cell-free amniotic fluid at 14 weeks gestation. Amniotic fluid from normal pregnancies has sufficient β-galactosidase activity to be measured by a fluorimetric assay. A range of 23.3 to 61.3 nmol per ml per hr was found for normal amniotic fluid in the period of 14 to 20 weeks gestation. The amniotic fluid from the affected pregnancy was found to have a complete absence of β-galactosidase activity. The activity of hexosaminidase in the same amniotic fluid sample was measured and a value of 590 nmol per ml per hr (normal: 244 to 644 nmol per ml per hr) was found. This second enzyme served as an internal control for the analysis. The diagnosis of G<sub>M1</sub>-gangliosidosis was confirmed by histologic examination of the fetus after termination of the pregnancy at 17 weeks.

A fluorimetric assay using 4-methylumbelliferyl-β-D-galactopyranoside as substrate has been used for the measurement of β-galactosidase activity in amniotic fluid. In normal amniotic fluid, the enzyme exhibits two pH optima at 4.5 and 5.9. The enzyme activity was found to increase slightly near term from a value of 3 nmol substrate hydrolysed per min per g protein to a value of 10 nmol substrate hydrolysed per min per g protein. Storage of the amniotic fluid for six months at −20°C caused a loss in activity of 30 to 40 percent.

**Pompe's Disease**

Pompe's disease is a fatal disorder caused by a deficiency of the lysosomal enzyme acid α-1, 4-glucosidase thus resulting in the accumulation of glycogen in the lysosomes of affected tissues. Symptoms manifest themselves during the first few months of life and the disease is characterized by cardiac enlargement and muscular weakness. The in utero diagnosis of Pompe's disease has been reported in the literature on the basis of a deficiency of α-1, 4-glucosidase in cell-free amniotic fluid. However, the same investigators found normal activity in the amniotic fluid from another affected pregnancy.

Glycogen is degraded in lysosomal structures by two isoenzymes. These two isoenzymes have different pH optima at 4 and 7. The isoenzyme absent in Pompe's disease has an optimum at pH 4 and affected patients show normal activity of the isoenzyme with an optimum of pH 7. The enzyme found in the amniotic fluid from both normal and affected pregnancies shows an optimum at pH 6, the same as found for an isoenzyme present in the kidney of both normal and affected patients. The kidney may be the source of the amniotic isoenzyme. The enzymes have similar properties except that the amniotic isoenzyme is inactivated by heating at 45°C while the kidney isoenzyme is stable at this temperature. The highest activity of the amniotic fluid enzyme is found in that fraction sedimented with the lysosomes by ultracentrifugation, apparently indicating that the enzymatic activity resulted from
the lysis of cells present in the amniotic fluid. However, cultured and uncultured amniotic fluid cells have revealed an isoenzyme similar to that found in liver cells and not the kidney type enzyme. The amnion was ruled out as the source of this isoenzyme.34

Although the prenatal diagnosis of Pompe's disease cannot be made by the direct analysis of cell-free amniotic fluid, it can be made by determination of enzyme levels in uncultured as well as cultured amniotic fluid cells.9

FUCOSIDOSIS

Fucosidosis is transmitted by an autosomal gene and results in the absence of activity of the enzyme α-L-fucosidase. The prenatal diagnosis of fucosidosis by measurement of the activity of α-L-fucosidase in cell-free amniotic fluid has been reported.20 The enzyme activity in fluid from an affected twin pregnancy at 14 and 18 weeks gestation was only 10 percent of the activity of a control sample. The diagnosis was confirmed after delivery by demonstration of an absence of α-L-fucosidase activity in white blood cells obtained from the identical twins. The activity of the enzyme α-D-mannosidase was measured in the same amniotic fluid as an internal control, and its activity was found to be slightly elevated.

I-CELL DISEASE

I-Cell disease is a mucopolysaccharidosis without mucopolysacchariduria and is classified as Type II mucolipidosis. It is characterized by the presence of coarse cytoplasmic inclusions in cultured fibroblasts and an Hurler-like syndrome. It is an autosomal recessive trait and death usually occurs between one and nine years of age.40 Most lysosomal enzymes, with the exception of β-glucosidase and acid phosphatase, show decreased activity in the tissues but increased activity in the body fluids of affected patients.45

The activity of acid hydrolase enzymes in the amniotic fluid of a fetus afflicted with I-Cell disease has been studied, and the prenatal diagnosis of I-Cell disease has been made.12 The activities of acid phosphatase and α-glucosidase were found to be normal. The activities of β-glucuronidase, arylsulfatase A, α-galactosidase, β-galactosidase, and hexosaminidase were all significantly elevated. The normal values in amniotic fluid for acid phosphatase3,38 α-glucosidase3,35,38 β-glucuronidase,3,13 α-galactosidase,30 β-galactosidase19 and hexosaminidase3,38 have been reported elsewhere. In that study of the affected pregnancy, a normal amniotic fluid sample from a pregnancy of the same gestational age was used as an external control and the analysis of acid phosphatase served as an internal control.

References

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